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Mechanisms of corticosteroid action on lymphocyte subpopulations

II. DIFFERENTIAL EFFECTS OF *IN VIVO* HYDROCORTISONE, PREDNISONE AND DEXAMETHASONE ON *IN VITRO* EXPRESSION OF LYMPHOCYTE FUNCTION

A. S. FAUCI *Laboratory of Clinical Investigation, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, U.S.A.*

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SUMMARY

The present study was undertaken to determine what, if any, differential effects various commonly used corticosteroid preparations had on the numbers and specific functions of lymphocyte subpopulations when these agents were administered in equivalent pharmacological dosages. Normal volunteers received a single dose of either 320 mg of hydrocortisone intravenously, 80 mg of prednisone orally, or 12 mg of dexamethasone orally. There was a marked lymphocytopenia and monocytopenia maximal 4–6 hr following administration of all three corticosteroid preparations with almost identical kinetics and degree of fall in total cell numbers as well as proportions of thymus-derived and bone marrow-derived lymphocytes. Hydrocortisone and prednisone caused only a slight suppression of phytohaemagglutinin (PHA) induced lymphocyte blastogenesis which could be reversed at supra-optimal concentrations of PHA. On the contrary, dexamethasone administration caused a marked suppression of PHA responses which was not reversed by supra-optimal PHA stimulation. In addition, hydrocortisone and prednisone administration did not suppress non-specific PHA-induced cellular cytotoxicity, while dexamethasone caused a marked suppression ($P<0.001$) of cytotoxicity. These studies show that although equivalent anti-inflammatory doses of these three corticosteroid preparations cause almost identical suppression of the numbers of circulating lymphocyte populations, they have a differential effect on certain *in vitro* functional correlates of cell-mediated immunity.

INTRODUCTION

Corticosteroids have been extensively employed as effective therapeutic agents in a variety of inflammatory or immunologically mediated diseases (Schwartz, 1968). These agents have been clearly demonstrated to cause several anti-inflammatory and/or immunosuppressive effects in man, including decreased migration of cells into inflammatory sites (Rebuck & Mellinger, 1953; Boggs *et al.*, 1964), circulating lymphocytopenia (Fauci & Dale, 1974, 1975a; Yu *et al.*, 1974; Webel *et al.*, 1974), and monocytopenia (Fauci & Dale, 1974, 1975a; Yu *et al.*, 1974), decreased immunoglobulin levels (Butler & Rossen, 1973) and impaired expression of cutaneous delayed hypersensitivity (Gabrielsen & Good, 1967). Several corticosteroid preparations of varying anti-inflammatory potency are commonly used in clinical practice today. When these various agents are administered in

Correspondence: Dr Anthony S. Fauci, Building 10, Room 11B–09, National Institutes of Health, Bethesda, Maryland 20014, U.S.A.

what is generally considered to be equivalent potency dosages, they are said to have similar anti-inflammatory properties (Liddle, 1961; Thorn, 1966). Reproducible specific lymphocyte-related phenomena following the administration of various doses of hydrocortisone (Fauci & Dale, 1974), prednisone (Yu *et al.*, 1974) or methylprednisone (Webel *et al.*, 1974) to normal volunteers as well as prednisone (Fauci & Dale, 1975a) to patients are a circulating lymphocytopenia, particularly of the thymus-derived (T) cell population, as well as a differential suppression of functional lymphocyte populations as measured by *in vitro* blastogenic response to mitogens and antigens (Fauci & Dale, 1974, 1975a). It is uncertain whether different corticosteroid preparations, even when given in so-called equivalent anti-inflammatory potency dosages, have the same or differential effects on the numbers and specific functions of circulating lymphocyte subpopulations. The present study was undertaken to determine any quantitative or qualitative differences in the effects of equivalent anti-inflammatory potency doses of three commonly used corticosteroid preparations (hydrocortisone, prednisone and dexamethasone) on the distribution and *in vitro* expression of certain functional capabilities of circulating lymphocyte subpopulations in normal humans.

MATERIALS AND METHODS

Subjects. Twenty-two normal adult volunteers (twelve men, ten women; ages 19–25 years) were studied. They were all in excellent health and were taking no medications during the time of the study. Since the effects of administration of a single dose of three separate corticosteroid preparations were studied in several assays and each preparation was studied in six to eight individuals, it was necessary to study two drugs in a few subjects at separate times. Those subjects who received more than one drug were given the drugs at least 2 weeks apart.

Treatment regimens. At 08·00 hr subjects received a single dose of either hydrocortisone (The Upjohn Company, Kalamazoo, Michigan) 320 mg intravenously (i.v.), prednisone (The Upjohn Company) 80 mg orally (p.o.), or dexamethasone (Merck, Sharp and Dohme, West Point, Pennsylvania) 12 mg p.o. These dosages are considered to be of equivalent anti-inflammatory potency (Sayers & Travis, 1971). Blood samples were drawn for white blood cell (WBC) and differential counts immediately prior to drug administration (0 hr) and 2, 4, 6, 8, 12, 24 and 48 hr following administration. In addition, 30-ml samples of heparinized blood were drawn at 0, 4, 24 and 48 hr for lymphocyte studies.

Total leucocyte and differential counts. Leucocyte counts were performed in a Coulter Counter (Model Fn, Coulter Electronics, Incorporated, Fine Particle Group, Hialeah, Florida). Differential counts were performed on peripheral blood smears stained with Wright's stain. Two hundred cells per smear were counted by the same observer throughout the study.

Preparation and culture of lymphocyte suspensions. Mononuclear cells (lymphocytes and monocytes) were obtained by Hypaque–Ficoll density gradient centrifugation (Böyum, 1968) of heparinized blood samples obtained at 0, 4, 24 and 48 hr following drug administration. The mononuclear cells were prepared and cultured as previously described (Fauci & Dale, 1975a). Quadruplicate cultures were done in Cooke microtitre plates (Cooke Laboratory Products, Cooke Engineering Company, Alexandria, Virginia). Each well contained 0.2 ml of cells in a concentration of $0·5 \times 10^6$/ml in Eagle's minimum essential media (MEM-S) (Grand Island Biological Company, Grand Island, New York) and 15% homologous AB serum. Cultures were incubated for 3 days at 37°C in 5% CO_2 in air, at 100% humidity with 10 μl of various concentrations of phytohaemagglutinin (PHA) HA16, lot K 9347 (The Wellcome Research Laboratories, Beckenham, Kent, England). The doses used were 0·5, 1·0, 2·0, 5·0 and 10 μg/ml of culture. Four hours before harvesting 0·4 μCi of tritiated thymidine (6·7 Ci/mM, New England Nuclear, Boston, Massachusetts) were added to each well. The cells were harvested from the wells onto fibreglass filters by a semi-automated microharvesting device, washed with 10% trichloroacetic acid (TCA) and 95% ethanol, and placed in 10 ml of Aquasol (New England Nuclear), and counted in a liquid scintillation counter (Model LS-350, Beckman Instruments, Incorporated, Fullerton, California). An entire PHA dose–response curve was done on the lymphocytes of each subject, at each time point (0, 4, 24 and 48 hr after drug administration), for each corticosteroid preparation tested. The arithmetic mean of the counts per minute (ct/min) of quadruplicate cultures was determined. In order to avoid any possible misrepresentation of data on the basis of corticosteroid-induced changes in baseline counts (unstimulated cultures), the degree of stimulation is expressed both as the difference in ct/min per 10^6 lymphocytes between stimulated and unstimulated (control) cultures (Δct/min), and as the stimulation index which is the ratio of ct/min of stimulated or experimental cultures to unstimulated or control cultures (E/C).

Circulating lymphocyte subpopulations. Thymus-derived (T) lymphocytes were identified as previously described (Fauci & Dale, 1974) by their ability to form spontaneous erythrocyte (E) rosettes with sheep red blood cells (SRBC) (Jondal, Holm & Wigzell, 1972). Two hundred lymphocytes were counted by the same observer throughout the study using phase contast optics at ×400 magnification on a Zeiss microscope (Carl Zeiss, Incorporated, New York). Lymphocytes binding three or more SRBC were considered positive.

Bone marrow-derived (B) lymphocytes were identified by their ability to bind sheep erythrocytes (E) coated with antibody (A) and complement (C) to form EAC rosettes (Bianco, Patrick & Nussenzweig, 1970). Rabbit IgM antibody-coated SRBC (EA) were generously supplied by Dr Michael M. Frank and were prepared as previously described (Frank & Gaither, 1970). Fresh mouse serum served as the source of complement (C) and the assay was performed as previously described (Fauci, 1975a). An attempt was made to distinguish monocytes from lymphocytes by morphology, and by their phagocytosis of latex particles. However, it becomes evident in doing the EAC assay that even with great care the margin of error can be wide and a variable percentage of lymphocytes may be identified as monocytes and vice versa. Hence, it is necessary to recognize that although the fraction of EAC rosetting cells represents predominantly B lymphocytes, a certain percentage of them may indeed be monocytes.

In each subject, the percentage of cells which formed E and EAC rosettes were added and the sum was subtracted from 100% to give the percentage of cells possessing neither surface marker. These cells will be empirically referred to as 'null' cells in the present study, although it is clearly recognized that a certain percentage of this small group of cells would probably be identified as either T or B lymphocytes if additional assays for other T- or B-cell surface markers were performed (Jondal, Wigzell & Aiuti, 1973).

PHA-induced cellular cytotoxicity. PHA-induced cellular cytotoxicity against radioactive chromium (^{51}Cr) labelled chicken erythrocyte target cells was assayed at 0, 4 and 24 hr after corticosteroid administration by a slight modification (Sherwood & Blaese, 1973) of a previously described method (Perlmann, Perlmann & Holm, 1968). Sterile chicken blood mixed with an equal volume of Alsever's solution (Flow Laboratories, Rockville, Md.) was stored at 4°C and used within 7 days of being drawn. Immediately prior to use, the cells were washed three times with phosphate-buffered saline, pH 7·4, and brought to a concentration of 10^8/ml in MEM-S with 10% foetal calf serum (FCS). 0·1 ml of this suspension was put in a 9·5 × 1·5 cm plastic tube and 0·1 ml of sodium chromate (Amersham/Searle Corporation, Arlington Heights, Illinois) (1 mCi/ml) containing 100 μCi or ^{51}Cr was added. The mixture was incubated at 37°C for 30 min with gentle agitation every 10 min. The cells were then washed three times at 4°C in MEM-S and reconstituted with 1 ml of MEM-S containing 10% FCS. Mononuclear cells were brought to a concentration of 1×10^6/ml in MEM-S containing 10% FCS. Cultures with and without PHA were performed in triplicate in 1 × 7·5 cm plastic tubes. Into each culture tube was added 1 ml of effectors (1×10^6/ml), either 10 μg PHA in 0·1 ml MEM-S or 0·1 ml of MEM-S and 1×10^6 ^{51}Cr-labelled chicken erythrocyte target cells in 0·1 ml. The cultures were incubated at 37°C in 5% C_2 in air and 100% humidity for 40 hr. The culture tubes containing a total volume of 1·2 ml were then spun at 1000 *g* for 10 min at 4°C and 0·6 ml of the supernate was pipetted into a separate tube. The 0·6 ml supernatant tube and the remaining 0·6 ml pellet were counted separately in an automatic gamma counter (Series 1185, Nuclear Chicago Corporation, Des Plaines, Illinois). The percentage of ^{51}Cr released by the erythrocyte target into the supernate was determined by the following formula (Sherwood & Blaese, 1973):

$$\text{percentage } ^{51}\text{Cr release} = \frac{\text{supernatant ct/min} \times 2}{\text{supernatant ct/min} + \text{pellet ct/min}} \times 100.$$

The degree of cytotoxicity is expressed as the percentage ^{51}Cr release in the presence of PHA minus the percentage ^{51}Cr release in the absence of PHA. In separate preliminary experiments, 1 μg and 100 μg of PHA were added to the cultures instead of 10 μg, cultures were incubated for 24 and 72 hr in addition to 40 hr, and the effector to target cell ratio was varied from 1:1 to 50:1. It was found that the most reproducible results with optimal cytotoxicity associated with least spontaneous target cell lysis occurred with the conditions employed in the present studies (effector to target cell ratio of 1:1 with 40 hr incubation). PHA-induced lymphocyte cytotoxicity against chicken erythrocytes has been shown to be independent of blast transformation (Perlmann & Holm, 1969), and was felt to be predominantly a T lymphocyte-dependent process (Möller, Sjöberg & Anderson, 1972; Kirchner & Blaese, 1973), although recent evidence has suggested that different populations of lymphocytes can mediate mitogen-induced cellular cytotoxicity (Muchmore *et al.*, 1975). In addition, other non-lymphocyte cell types such as monocyte–macrophages and neutrophils can function as effectors in this system (Perlmann & Holm, 1969).

RESULTS

Total lymphocyte and monocyte counts

The mean total lymphocyte and monocyte counts at various times after administration of a single dose of each corticosteroid preparation are shown in Fig. 1. Following ad-

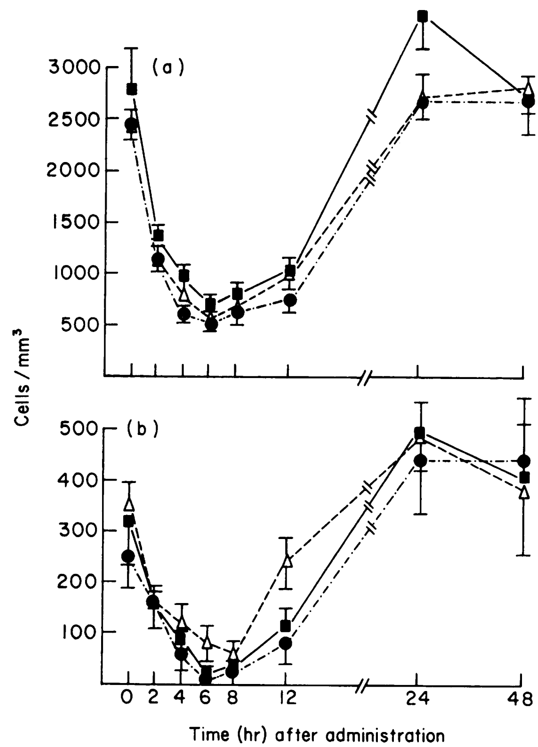

FIG. 1. The effect of corticosteroid administration on absolute circulating (a) lymphocyte and (b) monocyte counts. Subjects received a single dose of either hydrocortisone (■, 320 mg, i.v.), prednisone (△, 80 mg, p.o.) or dexamethasone (●, 12 mg p.o.). There were eight subjects in each treatment group. Each data point represents the mean (±s.e.m.) counts at various time intervals following corticosteroid administration.

ministration of each drug, there was a marked but transient lymphocytopenia and monocytopenia which was maximal at 4–6 hr. There was a return to normal lymphocyte and monocyte counts, and in some individuals a rebound to slightly supranormal counts by 24 hr, particularly with the monocyte counts. The degree of maximal lymphocytopenia and monocytopenia was the same for all three drugs tested, and the kinetics of the curves are quite similar for all three agents.

Lymphocyte subpopulations

The effect of administration of various corticosteroid preparations on lymphocyte subpopulations is shown in Table 1. For all three agents there was a highly significant decrease ($P<0{\cdot}001$, Student's *t*-test) in both E and EAC rosetting lymphocytes 4 hr after drug administration with a return to normal counts by 24 hr. Lymphocytes with neither the E nor EAC surface marker ('null cells') were slightly decreased by all three agents. This decrease was significant with dexamethasone ($P<0{\cdot}01$), but not with hydrocortisone ($P>0{\cdot}2$) or prednisone ($P>0{\cdot}2$).

Lymphocyte blastogenic responses to PHA

The effect of administration of each of the three corticosteroid preparations on the *in vitro* blastogenic response to stimulation with a wide dose range of PHA is shown in Fig. 2. Stimulation is compared at 0 hr and 4 hr which is the point of maximal lymphocytopenia. Both the Δct/min and the stimulation index (E/C) are shown. Hydrocortisone administration

TABLE 1. Effect of corticosteroid administration on lymphocyte subpopulations

	Time (hr)	Total lymphocyte E rosette (cells/mm³)	Total E rosette (cells/mm³)	Total EAC rosettes (cells/mm³)	Total 'null' cells (cells/mm³)
Hydrocortisone	0	2798 (±366)†	1935 (±232)	589 (±78)	273 (±115)
(320 mg, i.v.)	4	975 (±110)	565 (±50)	134 (±33)	143 (±57)
(n = 5)*	24	3817 (±359)	2617 (±215)	731 (±123)	468 (±98)
Prednisone	0	2393 (±102)	1612 (±74)	430 (±27)	345 (±96)
(80 mg, p.o.)	4	780 (±163)	438 (±71)	145 (±32)	197 (±78)
(n = 5)	24	2708 (+213)	1779 (+206)	557 (±79)	303 (±132)
Dexamethasone	0	2445 (±143)	1589 (±136)	480 (±25)	378 (±52)
(12 mg, p.o.)	4	612 (±77)	385 (±68)	83 (±6·4)	151 (±48)
(n = 6)	24	2680 (±198)	1796 (±152)	537 (±55)	345 (±88)

* n is the number of subjects studied in each treatment group.
† Mean (±s.e.m.).

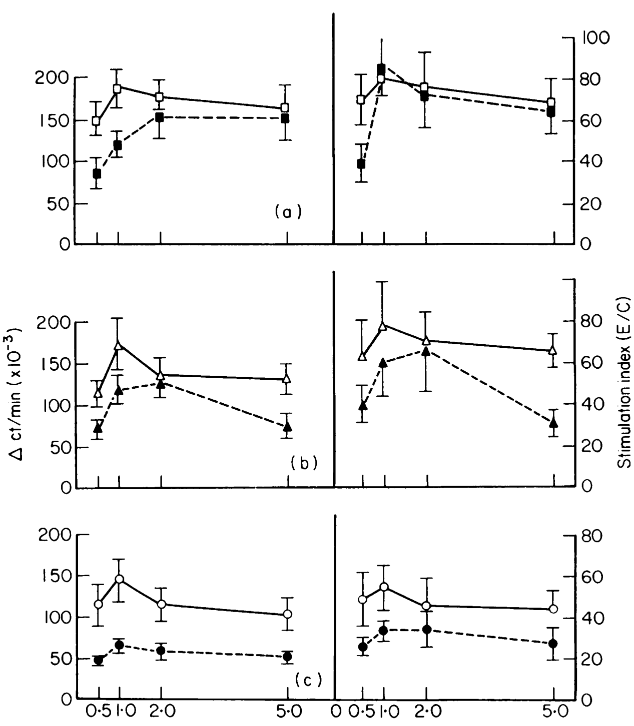

FIG. 2. The effect of corticosteroid administration on the *in vitro* lymphocyte blastogenic response to PHA. Subjects received a single dose of either hydrocortisone, prednisone or dexamethasone: (a) hydrocortisone 320 mg, i.v.: □, 0 hr; ■, 4 hr after hydrocorticosterone; (b) prednisone 80 mg, p.o.: △, 0 hr; ▲, 4 hr after prednisone; (c) dexamethasone 12 mg, p.o.: ○, 0 hr; ●, 4 hr after dexamethasone. There were six to eight subjects in each treatment group. Lymphocyte responses at 0 hr were compared with responses of equal numbers of lymphocytes at 4 hr following corticosteroid administration. Responses are shown as both the Δct/min and the E/C. Each data point represents the mean (±s.e.m.) responses to various *in vitro* concentrations of PHA.

caused a significant decrease in PHA stimulation (using Δct/min) at the lower concentrations of PHA ($P<0{\cdot}05$ for 0·5 and 1·0 μg/ml). However, the concentration curve shifted in the hydrocortisone-treated group at 4 hr such that peak response was seen at 2·0 μg/ml of PHA, and there was no significant difference in PHA stimulation ($P>0{\cdot}2$) at the higher concentrations of mitogen. Hence, the suppression by *in vivo* administration of hydrocortisone of the *in vitro* lymphocyte response to PHA could be overcome by higher stimulatory concentrations of PHA.

When the E/C was used to measure stimulation, hydrocortisone did not cause a significant suppression even at the lowest concentration of PHA ($P<0{\cdot}05$), and no suppression whatever at higher concentrations of PHA ($P>0{\cdot}2$). This finding of lack of suppression when E/C was used to express data, while suppression was seen when data was expressed by Δct/min is explained by the fact that suppression of the unstimulated or control (C) cultures together with suppression of the stimulated or experimental (E) cultures tends to keep the ratio E/C constant. On the other hand, suppression of gross ct/min would clearly be detected by a suppression of Δct/min.

Prednisone administration caused a slight but not a significant suppression of stimulation by PHA at all concentrations of mitogen except 5·0 μg/ml which was significantly suppressed ($P<0{\cdot}05$). Similar to hydrocortisone administration, prednisone administration caused a shift in the concentration curve of PHA stimulation to optimal stimulation at supramaximal concentrations of the mitogen. However, at the highest supramaximal concentration of PHA (5·0 μg/ml), blastogenesis was suppressed following prednisone administration. On the other hand, dexamethasone administration caused a significant suppression of PHA stimulation (using Δct/min) at all concentrations of mitogen ($P<0{\cdot}05$). When the data were expressed as E/C, there was a slight suppression of stimulation by all concentrations of PHA, but these changes were not significant. Hence, dexamethasone administration differs from hydrocortisone and prednisone administration in that dexamethasone suppressed PHA stimulation at all concentrations of the mitogen, while hydrocortisone and prednisone caused a shift in the concentration curve of PHA such that the suppression seen at sub-optimal and optimal stimulatory mitogen concentrations could be reversed at supraoptimal concentrations. By 24 hr the PHA responses of all three groups returned to normal and remained normal and unchanged at 48 hr.

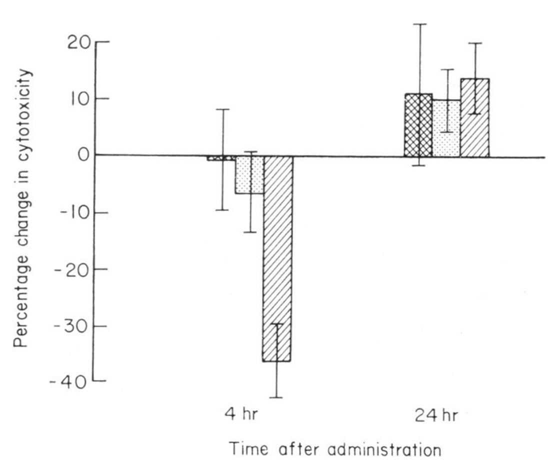

FIG. 3. The effect of corticosteroid administration on PHA-induced cellular mediated cytotoxicity. Subjects received a single dose of either hydrocortisone, prednisone or dexamethasone. There were six subjects in each treatment group. Data are represented as the mean (±s.e.m.) percentage change in cytotoxicity at 4 hr and 24 hr following corticosteroid administration compared to 0 hr. Cross-hatched columns, hydrocortisone, 320 mg, i.v.; stippled columns, prednisone, 80 mg, p.o.; hatched columns, dexamethasone, 12 mg, p.o.

PHA-induced cellular cytotoxicity

The effects of the administration of various corticosteroid preparations on the PHA-induced cellular cytotoxicity are illustrated in Fig. 3. At 4 hr following drug administration, hydrocortisone had no effect on the cytotoxicity of mononuclear cells remaining in the circulation at this time compared to the cytotoxicity of an equal number of mononuclear cells at 0 hr, while prednisone caused a minimal suppression of cytotoxicity. On the other hand, dexamethasone administration caused a marked suppression of cytotoxicity at 4 hr. There was a 36 ($\pm$6·5)% decrease in cytotoxicity at 4 hr following dexamethasone compared to 0 hr ($P<0{\cdot}001$). This effect is significantly greater than that caused by hydrocortisone or prednisone ($P<0{\cdot}02$). By 24 hr following drug administration, cytotoxicity rebounded to slightly above baseline for all three corticosteroid-treated groups.

DISCUSSION

It is generally held that there are no significant qualitative differences in the so-called general 'anti-inflammatory actions' of the various commonly used corticosteroid preparations (Liddle, 1961; Thorn, 1966; Sayers & Travis, 1971). There have appeared, however, suggestions of a qualitative difference between dexamethasone and other corticosteroid preparations based on a few reports of its superiority over these other preparations in experimental haemorrhagic (Fine, 1970) and endotoxin (Weil, 1970) shock. In addition, differences between various corticosteroid preparations in their ability to suppress host resistance to infection were shown to depend on chemical structure, while showing no correlation with the anti-inflammatory properties of the drugs (Fauve & Pierce-Chase, 1967). More recently, striking qualitative differences in the effects of administration of prednisone and dexamethasone on localized leucocyte mobilization in man have been demonstrated (Peters *et al.*, 1972).

In the present study, the relative effects of administration of a single dose of hydrocortisone, prednisone, and dexamethasone in equivalent 'anti-inflammatory' dosages on numbers and functions of lymphocyte subpopulations were investigated. A remarkable similarity in the degree and the kinetics of the resulting lymphocytopenia and monocytopenia (Fig. 1) as well as in the effect on the numbers of circulating lymphocyte subpopulations (Table 1) was noted following administration of each of the three agents. The administration of dexamethasone, however, resulted in a greater suppression of PHA responsiveness of the lymphocytes remaining in the circulation at the point of maximal lymphocytopenia than did the administration of hydrocortisone and prednisone (Fig. 2).

An even more striking difference in these corticosteroid preparations was seen in the effects on PHA-induced cellular cytotoxicity (Fig. 3). In this system, dexamethasone administration was highly suppressive, while hydrocortisone and prednisone were not.

Thus, this study demonstrates a clear dichotomy between the remarkably similar suppression of the absolute numbers of circulating lymphocyte subpopulations and monocytes by these three agents and the marked suppression of the expression of certain lymphocyte functional capabilities seen following dexamethasone administration in contrast to little, if any, suppression of these functional capabilities seen following hydrocortisone and prednisone administration. A possible explanation of this dichotomy becomes apparent if one considers various mechanisms of corticosteroid-induced suppression of lymphocyte functions. It has been demonstrated in animals and man that the lymphocytopenia following corticosteroid administration is due to a redistribution of lymphocytes from the circulation to other body compartments (Fauci & Dale, 1975a, b; Cohen, 1972; Claman, 1971; Fauci, 1975a). It is particularly noteworthy that in man similar degrees and kinetics of maximal lymphocytopenia are seen following a single dose of a wide dosage range of a variety of preparations of varying plasma half-lives, and given by different routes of administration

(Fauci & Dale, 1974, 1975a; Yu *et al.*, 1974; Webel *et al.*, 1974). It appears that there is a lower limit (approximately 20% of pretreatment level) to the degree of maximal transient lymphocytopenia following a single dose of corticosteroid, regardless of the magnitude of dose, route of administration or plasma half-life of the preparation (Fauci, 1975b). It is not surprising then that the three preparations used in the present study gave almost identical pictures of monocytopenia and lymphocytopenia (Fig. 1).

The duration of the agent in the circulation, however, may be important in determining the effect on various lymphocyte functions. Despite the fact that the three preparations used in the present study are of equivalent anti-inflammatory potency, they possess quite different plasma half-lives (Sayers & Travis, 1971). Hydrocortisone and prednisone are 'short-acting' with plasma half-lives of approximately 80 min and 60 min respectively, while dexamethasone is relatively long-acting with a half-life of approximately 200 min. The importance of corticosteroid plasma half-life in the effect on lymphocyte function is strongly supported by recent studies in guinea-pigs (Balow, Hurley & Fauci, 1975) in which a single intraveneous dose of a soluble preparation of hydrocortisone, causing only a transient elevation of plasma cortisol, resulted in the same degree of lymphocytopenia as the intramuscular administration of a depot preparation of cortisone acetate which resulted in sustained elevation of plasma cortisol. However, the hydrocortisone did not suppress the functional capabilities of the lymphocytes remaining in the circulation, while the cortisone acetate markedly suppressed antigen-induced lymphocyte blastogenesis and macrophage inhibitory factor production.

In the present study it is uncertain whether the suppression of lymphocyte functional capabilities by dexamethasone and not by hydrocortisone and prednisone was due to a true qualitative difference in the effect of dexamethasone on the expression of lymphocyte function, a quantitative difference in suppression of function caused by the longer half-life of dexamethasone, or a selective depletion of monocytes and certain lymphocyte subpopulations by dexamethasone and not hydrocortisone and prednisone. The latter hypothesis is unlikely in light of the similarity in depletion of monocytes and lymphocyte subpopulations by the three agents as shown in Fig. 1 and Table 1. It is impossible to distinguish between the first two hypotheses, but data from animal studies (Balow *et al.*, 1975) suggest that sustained elevated plasma levels of corticosteroids are necessary for suppression of various lymphocyte functions.

Although many of the precise mechanisms of the corticosteroid effects observed in this study remain uncertain, it is quite clear that as various lymphocyte subpopulations are identified and characterized by markers as well as functional capacities, and as the effects of administration of various corticosteroid preparations on these subpopulations are further studied, one can no longer accurately extrapolate the concept of 'equivalent anti-inflammatory potencies' which were originally based on crude assays such as inhibition of granuloma formation around implanted cotton pellets in rats (Lerner *et al.*, 1964). When measuring corticosteroid-induced lymphocyte suppression, one must refer more specifically to relative effects of various corticosteroid preparations on the numbers, kinetics, and functional capabilities of lymphocyte subpopulations based on dose, dose interval, and duration of action of the drug.

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